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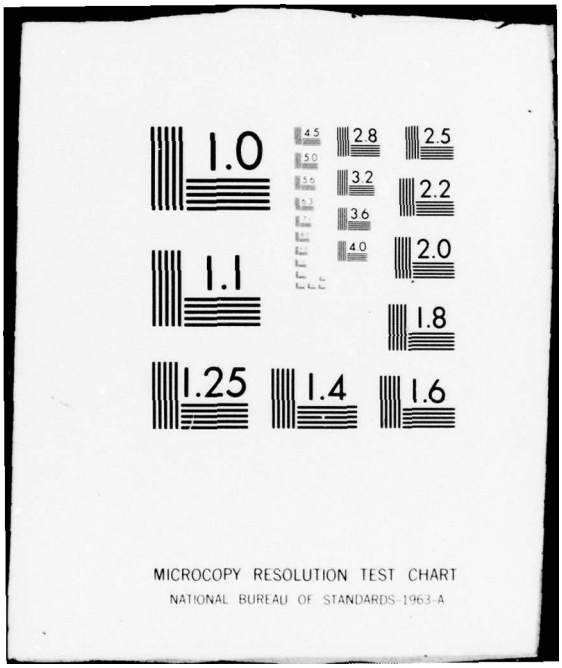
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A PORCINE BIOASSAY STUDY OF THE PHYSIOLOGICAL EFFECTS OF FIBER AND DYE DEGRADATION PRODUCTS (FDP) ON BURN WOUND HEALING

(9) Final rept.,

By

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Upon exposure to the thermal environment of an aircraft fire, many fire retardant fabrics off-gas fiber and dye degradation products (FDP). Condensation of these products on human skin raises questions concerning possible deleterious effects on burn wound healing. A porcine bioassay was used to study the physiological effects of FDP. Selected areas of living skin, protected by dyed aromatic polyamides and polybenzimidazole fabrics, were exposed to a thermal source adjusted to simulate a

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PREFACE

The vivarium of the United States Army Aeromedical Research Laboratory (USAARL) is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The animals used in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and AR 70-18. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

All authors were research investigators at the USAARL during the conduct of the experiments described herein.

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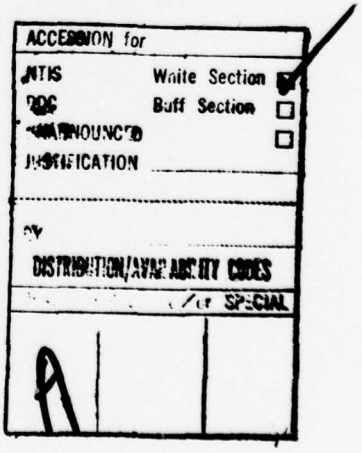

SUMMARY

Upon exposure to the thermal environment of an aircraft fire, many fire retardant fabrics off-gas fiber and dye degradation products (FDP). Condensation of these products on human skin raises questions concerning possible deleterious effects on burn wound healing. A porcine bioassay was used to study the physiological effects of FDP. Selected areas of living skin, protected by dyed aromatic polyamides and polybenzimidazole fabrics, were exposed to a thermal source adjusted to simulate a postcrash JP-4 fuel fire. Burn sites contaminated with FDP were evaluated by clinical observation and histological techniques. Healing of the burn wound was followed by recording time to begin epithelialization, time to closure of an open wound, and the amount and type of cicatrix formation. The experiment showed that each fabric has unique off-gasing products. The greatest amount of FDP was deposited on the skin when the skin was covered by a single layer of shell fabric separated by a 6.35 mm air gap. The presence of an intervening cotton T-shirt decreased the amount of FDP deposited on the skin. We found no evidence that FDP caused alterations in wound healing.

APPROVED: Stanley C. Knapp

STANLEY C. KNAPP
Colonel, MC
Commanding

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INTRODUCTION

Fire retardant fabrics worn by aviators offer protection from thermal insults. However, during the process of conflagration of these fabrics, fiber and dye degradation products (FDP) are released. The condensation of these products was apparent on the manikins (Figure 1) used in fire pit testing of flight suits and on pigskin used in bioassay testing of thermal protective underwear,[1] raising the question of whether FDP are beneficial or detrimental to the healing of a burn injury. A bioassay determination of thermal protection afforded by four candidate flight suit fabrics gave us the opportunity of addressing this question,[2] and this report describes the clinical observations and histological determinations used to evaluate FDP on burn wound healing.

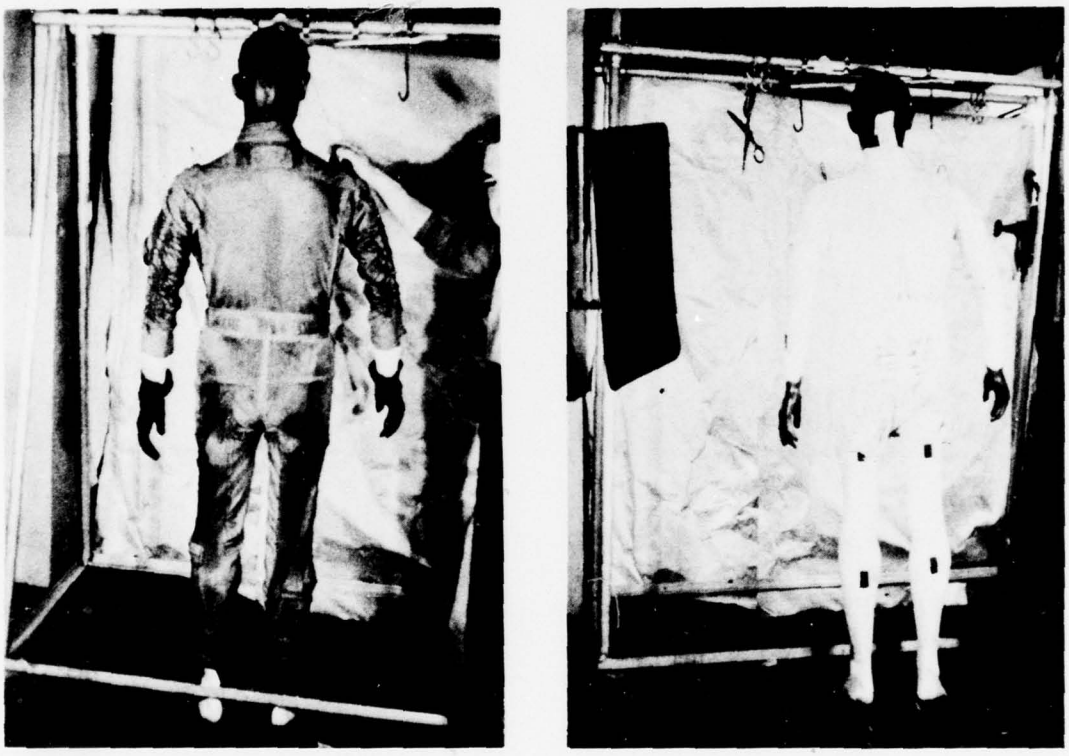

FIGURE 1. Manikin After Fire Pit Test Showing FDP On The Manikin Legs and Arms As Well As On The Cotton T-Shirt And Shorts. For more dramatic color photographs showing FDP on manikins, see Stanton, et al.[3]

METHODS AND MATERIALS

Twenty white domestic swine weighing 44 ± 7 kg were procured, quarantined, freed of internal and external parasites, and verified to be healthy prior to use in this study. The pigs were randomly assigned to one of four fabric configuration groups of five animals each (Table 1).

TABLE 1
FABRIC CONFIGURATION GROUPS

Group	Layers	Configuration	Air Gap
I	Single	In contact with skin	None
II	Single	Air gap between shell fabric and skin	6.35 mm
III	Double	Shell fabric in contact with T-shirt fabric in contact with skin	None
IV	Double	Air gap between shell fabric and T-shirt fabric with T-shirt fabric in contact with skin	6.35 mm

The swine were fasted overnight, premedicated with atropine (0.04 mg/kg) and fentanyl-droperidol (0.1 ml/kg), intubated and anesthetized with halothane, USP.[4] All the hair was closely clipped with a #40 clipper head.[5] The anesthetized pigs were placed on a rolling animal carriage with a pneumatically operated shutter system.[1,2] Each animal in the group received four separate exposures of five seconds duration to a standardized thermal source which delivered 3.07 ± 0.16 calories per square centimeter per second (70-90% radiative)[2] while protected by a template containing five exposure sites. Four of these sites were covered with one of the four test shell fabrics (Table 2, page 3), and one was a nonfabric control site (Figure 2, page 4). Thus, each shell fabric received 20 replications in each configuration and appeared in each separate exposure site according to a Latin Square randomization.

TABLE 2
EXPERIMENTAL FABRICS AND FDP

Fabric Code	Fabric Characteristics	FDP*
AFN	4.8 oz twill weave Nomex® aramide	H_2O, CO_2, CO, HCN, CH_4 ethylene, acetylene, benzene, hydrocarbon fragments, 1-3 dicyanobenzene, m-aminobenzonitrile, m-phenylene diamine, amide fragments
PBI	4.5 oz stabilized twill weave polybenzimidazole	H_2O, CO_2, CO, HCN, CH_4, SO_2, NH_3 ethylene, acetylene, hydrocarbon fragments, aryl and alkyl sulfates and sulfanates, NH salts
HT4	4.8 oz plain weave experimental high temperature polymer	H_2O, CO_2, CO, HCN, CH_4, NH_3, ethylene, acetylene, benzene, hydrocarbon fragments, 1,4-dicyanobenzene, p-aminobenzonitrile, p-phenylenediamine, 4-aminobenzanilide, phosphates
NWN	4.6 oz plain weave Nomex® aramide	H_2O, CO_2, CO, HCN, CH_4, acetylene, benzene, hydrocarbon fragments
/T	100% cotton T-shirt	Products unknown
/S	Air Gap (6.35 mm)	N/A

*Analysis of mass spectrometry data is more accurate if the initial chemical compositions of the fabrics and dyes are known before submitting them to pyroanalysis. The rate, amount, and content of gases and condensables that are released are dependent on the temperature, ΔT, atmosphere, and duration of exposure.

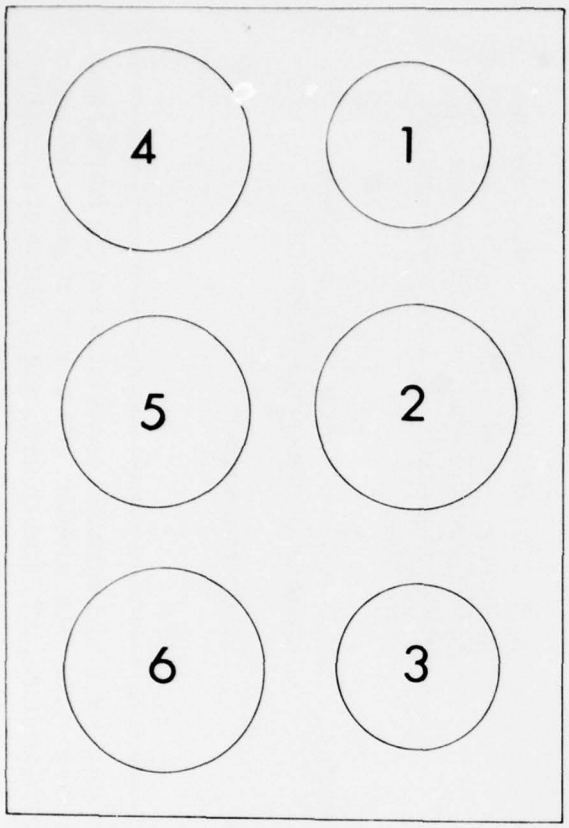

FIGURE 2. Template Hole Positions. An associated study required that holes 1 and 3 be smaller than the rest. Hole size did not influence data presented in this paper. For a complete description of template configuration, see Knox, et al.[2]

The resultant burns were evaluated immediately and at 24 hours postburn using photographic techniques and clinical observations for documentation. A grade was assigned based on the surface appearance and characteristics of the burn immediately and 24 hours postburn.[2] Frozen sections were made from some of the biopsies immediately postburn and submitted for hematoxylin and eosin (H&E) and unstained preparations. A Goulian dermatome was used to remove a split thickness skin graph (0.20 mm thick) on several of the test sites. Under anesthesia, incisional biopsies (4 mm wide and extending from the most severe portion of the burn area into the normal tissue outside the burn site)[1] were taken from each burn site at 24 hours and submitted in formalin for histological examination. Hematoxylin and eosin (H&E) stained sections were evaluated for general assessment of damage, dye deposition and actual depth of burn. The clinical observations were continued on selected animals for an extended period of time to evaluate

the initiation of epithelialization of the burn wound, contraction of the open biopsy wound, time to wound closure, and the amount and type of cicatrix formation.

RESULTS

Varying amounts of FDP were deposited on the burn wound depending upon the fabric and the fabric configuration (Figure 2, page 4, and Figures 3-6, pages 6-9). Group II appeared to have the greatest amount of visible FDP deposition on the burn wound by photographic (Figure 4, page 7) and clinical evaluation. Group III (Figure 5, page 8), Group IV (Figure 6, page 9), and Group I (Figure 3, page 6) demonstrated respectively less condensation of visible off-gasing products. The amount of visible FDP was reduced or absent 24 hours postburn (Figure 7, page 10) and was always absent after one week. In burns which formed steam blebs, the visible FDP were deposited only on the bleb and not on the underlying burned dermis (Figure 8, page 11). In all cases, the visible dye could be washed away with water or removed with the dermatome.

Unstained frozen section biopsies showed the dye deposited on the keratin layer or superficial epidermis (Figure 9, page 11). No dye penetrated to the base of the epithelial cells in any histological preparation. Formalin fixed biopsy specimens did not show FDP on either stained or unstained preparations.

In unburned skin, epithelialization was first observable on the fifth to seventh day post biopsy. It was first observable on the sixth to ninth day post biopsy in the burned skin. Contraction appeared to be the same for all biopsy sites. Biopsy incisions healed more rapidly in unburned skin (16-18 days) than in the burned skin (22-28 days). Burn damage was not as severe under any of the fabric samples as it was for the control sites. The control sites were the slowest to heal. There was no clinical difference in healing of the wounds contaminated with FDP when compared to the control sites. The thickness and type of eschar formation among the control sites and the burn sites protected by fabrics were clinically the same.

No wound infections were observed. No major trauma occurred to any of the animals or burn sites, although minor trauma probably occurred from contact with the concrete run and chain link fence which housed the animals.

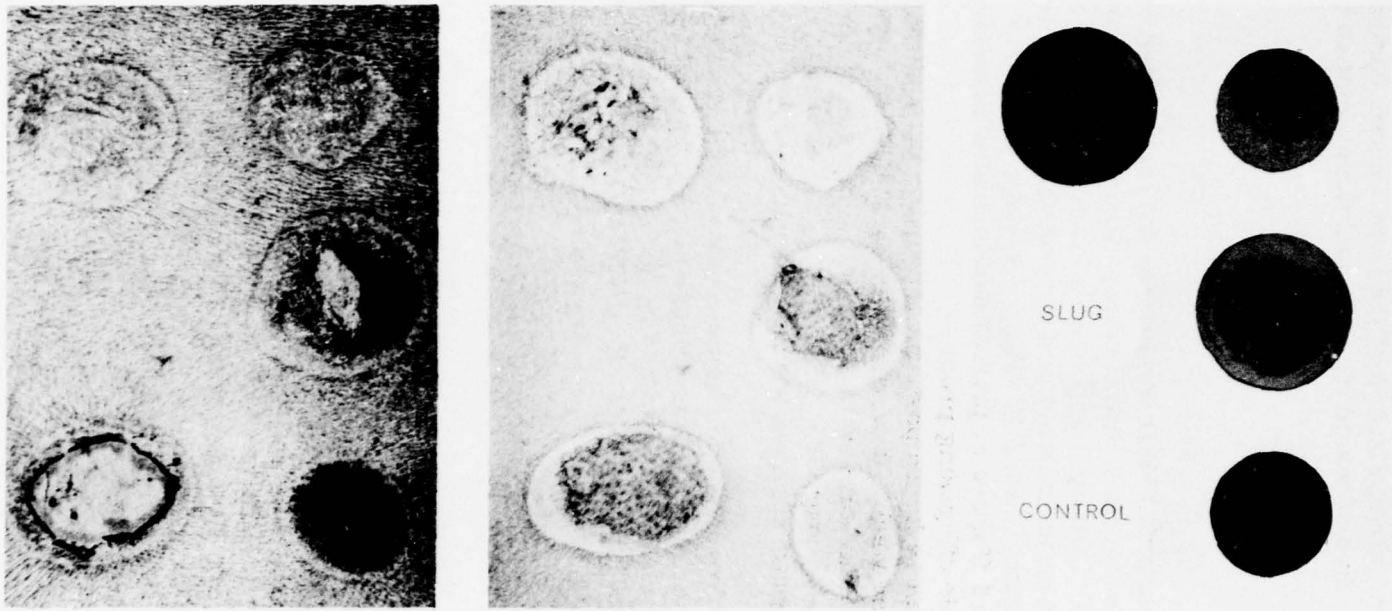

FIGURE 3. Group I (Single Layer in Contact With Skin). A. Immediately Postburn; B. 24 Hours Postburn; C. Fabrics: 1-NWN, 2-HT4, 3-PBI, 4-AFN, 5-Slug Calorimeter, 6-Control.

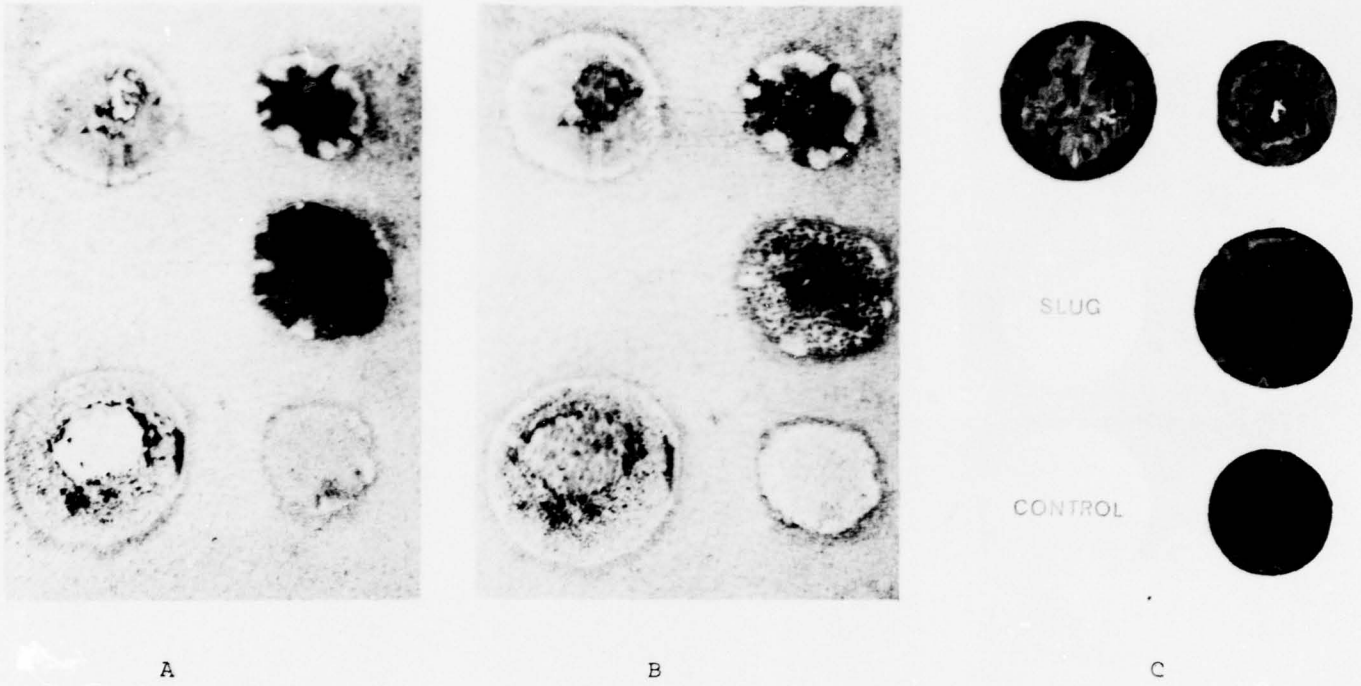

FIGURE 4. Group II (Single Layer With 6.35 mm Air Gap Between Fabric and Skin). A. Immediately Postburn; B. 24 Hours Postburn; C. Fabrics: 1-NWN/S, 2-HT4/S, 3-PBI/S, 4-AFN/S, 5-Slug Calorimeter, 6-Control.

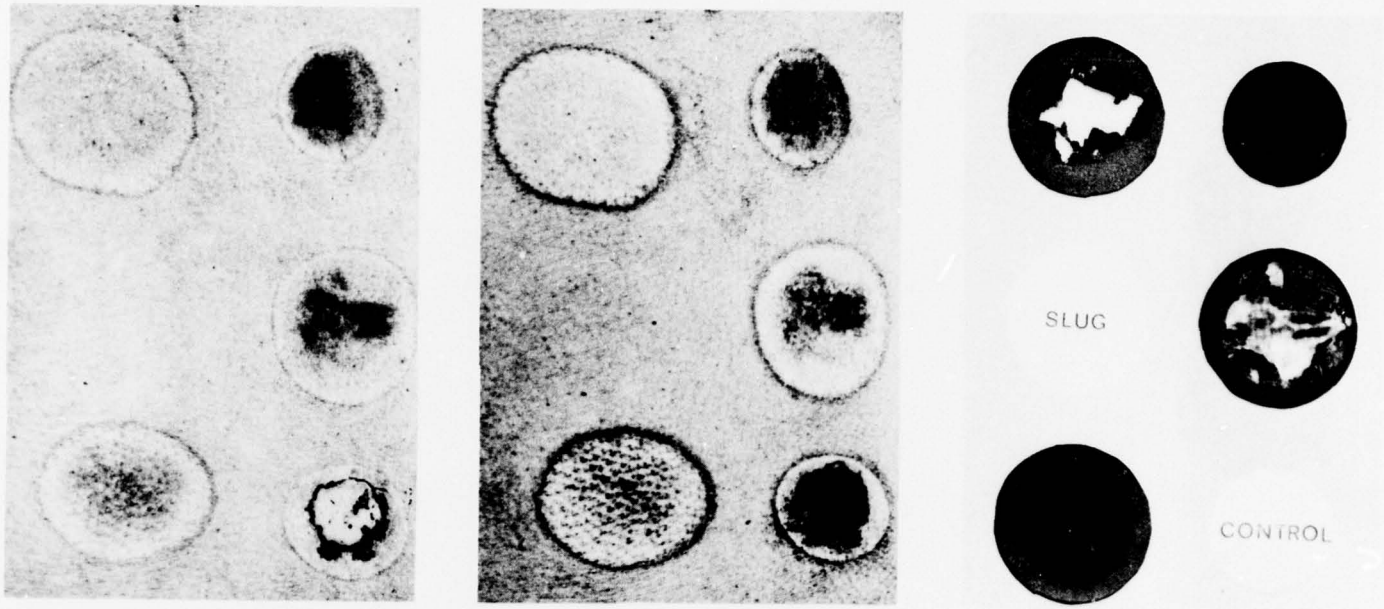

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FIGURE 5. Group III (Shell Fabric in Contact With T-Shirt in Contact With Skin). A. Immediately Postburn; B. 24 Hours Postburn; C. Fabrics: 1-PBI/T, 2-AFN/T, 3-Control, 4-NWN/T, 5-Slug Calorimeter, 6-HT4/T.

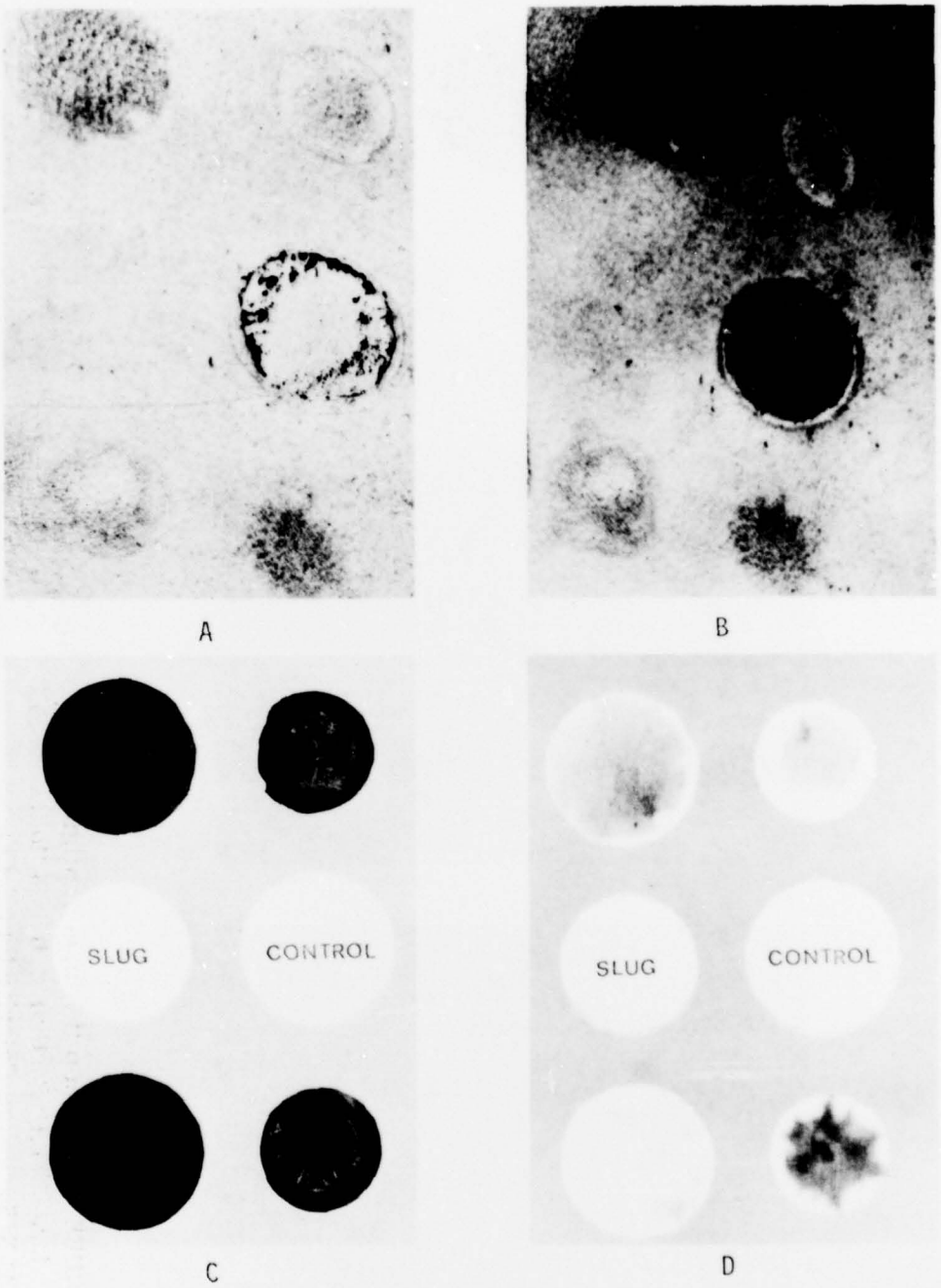

FIGURE 6. Group IV (6.35 mm Air Gap Between Shell Fabric and T-Shirt in Contact With Skin). A. Immediately Postburn; B. 24 Hours Postburn; C. Fabrics: 1-AFN/T/S, 2-Control, 3-NWN/T/S, 4-HT4/T/S, 5-Slug Calorimeter, 6-PBI/T/S; D. FDP on T-Shirt.

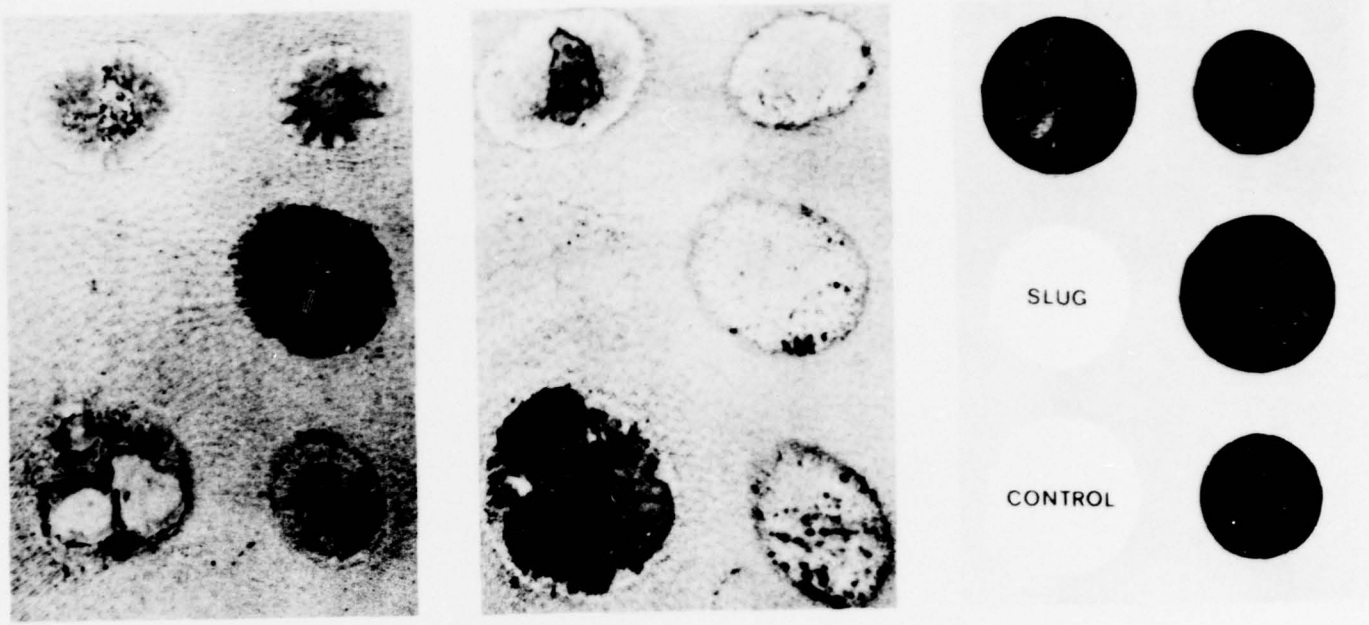

FIGURE 7. A Group II Fabric Configuration Showing Great Reduction of FDP at 24 Hours Postburn; A. Immediately Postburn; B. 24 Hours Postburn; C. Fabrics: 1-NWN/S, 2-HT4/S, 3-PBI/S, 4-AFN/S, 5-Slug Calorimeter, 6-Control.

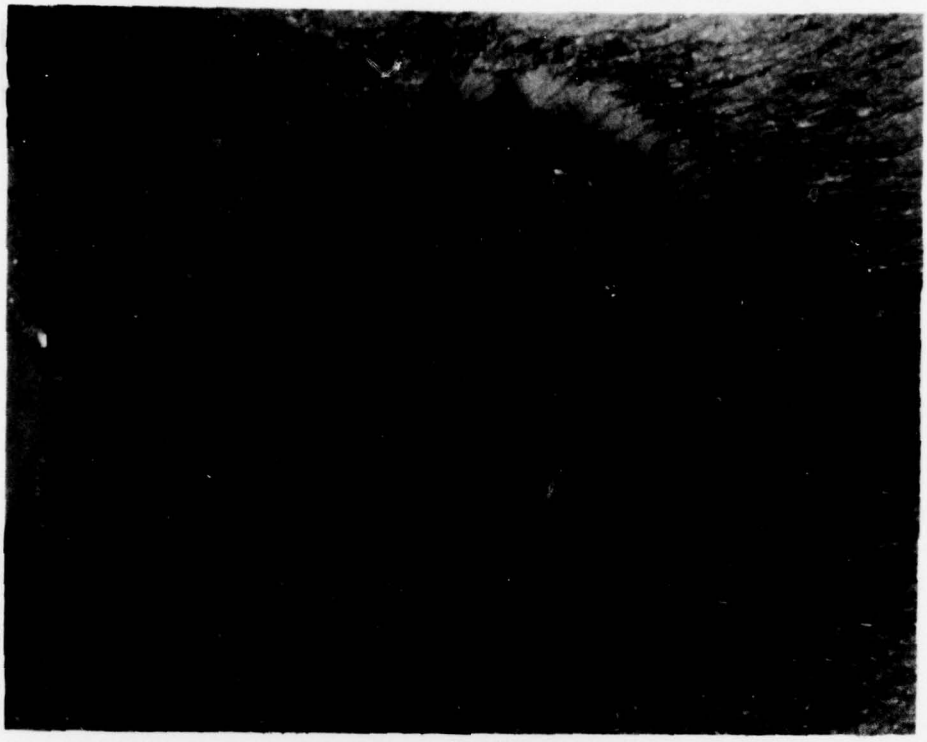

FIGURE 8. Visible FDP is deposited only on the steam bleb and not on the underlying burned dermis. Close-up of **Figure 4A, Position 4, 1.75X magnification.**

FIGURE 9. Photomicrograph (X50) of FDP As It Appeared On An Unstained Frozen Section. The separation is an artifact. Arrow 1 points to the dye layer, and arrow 2 points to the epithelial layer (H&E stain).

DISCUSSION

Under the conditions of our experiment, the visible components of the fiber and dye degradation were deposited on the epidermis (Figures 8 and 9, page 11). Microscopic examination of frozen sections of the wound immediately postburn showed dye particles present only on the keratin layer or superficial layer of epithelium. The split thickness skin graft (0.20 mm) showed no dye in the dermis. Mass spectrometry of the epidermis and dermis would have differentiated the depth of the dye deposition, but these studies were not performed because of their unavailability at the time of the experiment. The dye particles were not present on the stained biopsy specimens. This may be a result of their solubility during the fixation or staining process (immediate frozen section or 24 hours postburn formalin fixation). Some of the FDP were mechanically removed by simple water washing. All our evidence supports the hypothesis that FDP were deposited on the superficial surface of living skin during the thermal exposure, and this superficial location contributed to the disappearance of FDP after 24 hours in most animals.

Wound healing is a complex process which depends on such things as skin thickness, skin structure, skin chemistry, collagen formation, type and depth of damage, presence or absence of infection, trauma, and presence of toxic substances which act at either cellular or systemic levels.[6] It is not our purpose to describe in detail the normal healing process of wounds or burn injuries. Nonetheless, the major healing processes need to be addressed when differentiating the effect of topical agents.

Skin is a highly complex compound organ containing multiple structures derived from several germ layers. If natural processes are not disturbed, skin will restore its surface continuity by epithelialization, contraction, and synthesis of dense collagen tissue.[6] The alteration of skin physiology that results when simple eschar replaces complex tissue is of paramount importance but difficult to measure. The process of healing is the result of cell movement, cell division and synthesis of various proteins, all of which are basic biological processes.[6]

Hunter[7] and Peacock[6] have stated that studies of cutaneous wound healing can be accomplished by careful clinical observation. In addition, this repair process can be studied by histological and histochemical techniques, autoradiography and scanning electron microscopy. Alone, each of these techniques have shortcomings but in combination offer a reasonably complete analysis of wound healing.[8] Open wounds such as those created by

our biopsies provided the opportunity to study contraction. Our experiments combined clinical observation and histological techniques to study the normal healing process and the effects of FDP on wound repair.

Since epithelialization, contraction and formation of new connective tissue are all complex biological processes involved in wound healing, no single one will adequately describe this process.[8] For a wound to be healed by a surgeon's criteria, it must be resurfaced, continuity must be restored, and strength must be achieved.[8] The first two criteria apply to the open wound. Restoration of continuity is primarily a function of connective tissue. The depth of the wound, whether to deep fascia or panniculous carnosum, is of importance to experimental results.

There are differences of a quantitative nature in the healing of wounds between species. One must be cautious in transferring the results obtained in animals to the problems of wound healing in humans. Nevertheless, the basic mechanisms involved appear to be essentially the same for all species. Differences in healing rate usually can be explained by known metabolic and anatomical differences between species.[6]

Peacock states that collagen will denature at temperatures greater than 60° C and create a burn wound described pathologically as coagulation necrosis.[6] Stoll and Greene[9] state that the severity of skin burns depends on elevation of the skin temperature above a threshold of 44° C. This process proceeds logarithmically as the temperature increases. At 50° C, the damage rate is 100 times the rate at 45° C. The complexity of the healing process for burn injury makes designing a sensitive protocol for assessing burn wound healing very difficult. An even more complex problem arises when a burn injury is studied to determine whether substances applied topically add inhibitory factors to an already compromised wound healing situation. Our porcine model employs the burn wound as a basis for studying topical agents and uses the same pigskin in the immediate proximity to serve as an unburned and burned control. We know of no other model designed to study both aspects at the same time.

Other models have been used to study the systemic and local inhibitors of incisional (nonburned) wound healing. A rabbit ear model was used to study the effect of silver nitrate and sulfamylon on incisional wound healing.[10] In that experiment, an incisional skin wound was created that could not heal by contraction. Healing was assessed by elapsed time before epithelialization and elapsed time for complete wound healing. Their clinical assessment showed that new epithelium was present in five days when silver nitrate,

saline, or ointment base controls were used. When sulfamylon was used, epithelialization took eight days. Complete healing was present in 14 days in the silver nitrate and control groups and 28 days in the sulfamylon groups. In a more sophisticated adult guinea pig model, an open wound was used to study the effect of topical sulfamylon. Clinical observation, wound contraction rate, histology, autoradiography and hydroxyproline content determinations were used to assess the healing of the open wound.[11] These studies showed that sulfamylon significantly inhibited wound healing.

Painting FDP on open incisional (nonburned) wounds would be another means of assessing the effects. A variation of this study has been reported wherein the effect of rosaniline dyes on wound infection in guinea pigs was assessed.[12] In this case, triaminotriphenylmethane appeared to be beneficial while diaminotriphenylmethane lacked therapeutic value.

From our observations we were unable to show that FDP prolonged the time to epithelialization, interferred with normal contraction, or increased the time for wound closure. The amount, thickness, and type of eschar formation were not altered by the fiber and dye degradation products. The burn wounds followed the normal pattern of healing consistent with the severity of the burn. In no case could we determine that the FDP caused increased thermal damage.

In addition to possible local cytotoxic effects of FDP, there are well established systemic toxicities from some FDP, e.g., HCN and Aniline (Table II, page 3). In view of the apparent superficial location of the FDP and the usual massive exudation in severe burns, absorption could be expected to be minimal. However, the amounts of these substances available for absorption and their absorptivity through burned skin or via other routines in a burned victim have not been established. Studies have shown that cellular poisons such as cyanide and dinitrophenol will inhibit the movement of the wound edges.[6] The absorbed dose necessary for a toxicological effect would probably exceed that produced by the conflagration of an entire flight suit, which by itself would result in lethal injury. Therefore, from the clinical point of view, we believe that the degree of thermal protection afforded by the thermal protective fabric is much more important than the possible minor alterations in wound healing caused by fiber and dye degradation products.

CONCLUSIONS

Visible components of fiber and dye degradation products were deposited on the surface of burned skin. A single layer of fabric with a 6.35 mm air gap between it and the skin deposited the greatest amount of FDP. The presence of an intervening cotton T-shirt decreased the amount of FDP deposited on the skin. We found no evidence that the FDP caused alterations in wound healing.

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